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Body Polarity and Mineral Selectivity in the Demosponge *Chondrosia reniformis*

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Abstract. The skeleton of the common Mediterranean demosponge *Chondrosia reniformis* lacks endogenous spicules; but exogenous siliceous material is selectively incorporated into its collagenous ectosome, strengthening this layer. Nevertheless, the settling of sponge buds during asexual reproduction necessitates an active incorporation of the calcareous substratum through the sponge lower ectosome. This fact suggests the presence of a polarity in the sponge, with the lower surface selecting primarily carbonates, and the upper surface selecting exclusively silicates and quartz. Our observations under experimental conditions showed that the strong selectivity of the upper ectosome is realized only when the sponge is fixed to the substratum; if detached, the sponge incorporates both quartz and carbonates. In laboratory experiments, the incapacity of both kinds of ectosome to regenerate into a new complete sponge suggests that this polarity arises early in ontogeny.

Introduction

Chondrosia reniformis is a cushion-shaped, Atlanto-Mediterranean demosponge that usually lives on shallow rocky bottoms. A section through the sponge reveals two distinct regions: a cortical zone called ectosome, and an internal zone, the choanosome, which contains the choanocyte chambers. The ectosome is composed of a layer of flattened cells, exopinacocytes, that surround dense interwoven bundles of fibrils of collagen. In many circumstances the pinacocyte layer is loose, and the collagen fibrils can be in direct contact with water (Garrone *et al.*, 1975).

C. reniformis lacks the opaline spicules that are the main constituents of the skeleton of other demosponges; rather, the collagenous ectosome is strengthened by sand grains and exogenous spicules, which are actively incorporated by the sponge (Bavestrello *et al.*, 1995). Studies of foreign matter incorporation have been carried out on the sponge *Dysidea etheria.* In this species, the particles are incorporated by contraction of the dermal membrane, which probably separates or disrupts the thin exopinacocyte layer on the dermal membrane surface (Teragawa, 1986a, b). The ectosome of *C. reniformis* behaves similarly (Bavestrello *et al.*, in press).

In *C. reniformis,* the upper ectosome is able to select the minerals that settle on the sponge: thus, siliceous material is engulfed while the calcareous fragments that are the main sediments available in the surrounding environment are eliminated (Bavestrello *et al.*, 1996). In contrast, however, *C. reniformis* settles on calcareous rocks through the partial incorporation of outgrowths of this substratum by the lower ectosome. This process suggests a polarization in the sponge body, with a specificity for the incorporation of minerals that varies from the lower ectosome to the upper one.

Indeed, the polarization of the adult sponge body was already demonstrated 45 years ago with *Sycon raphanus.* When specimens of these sponges were bisected transversely, both halves could develop a complete new animal with the same polarity, from osculum to base, as the original (Tuzet and Paris, 1963).

The polarization of sponges relative to their position on the substratum probably arises in the larval stage. In

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the amphiblastula larvae of Calcarea, the flagellate cells of the anterior pole make the initial contact with the substratum (Bergquist and Green, 1977). In Demospongiae, several authors have suggested that the coeloblastula or parenchymella larvae express an existing polarity in their attachment to the substrate. But such views are highly speculative, because distinguishing between an anterior and a posterior hemisphere in these animals is difficult (see Simpson, 1984, for a review).

The aims of this work are to verify—through laboratory experiments and scanning electron microscopy (SEM)—the capacity of both kinds of ectosome of *Chondrosia reniformis* to develop specificity towards siliceous and calcareous materials, and to demonstrate the cellular basis of that specificity.

Materials and Methods

Along the rocky cliff of the Portofino Promontory (Ligurian Sea, Italy) *Chondrosia reniformis* lives from the surface to the base of the cliff (about 50 m depth). The specimens used in this study were collected during January 1997, at 10 m depth, on calcareous substrates.

We performed our experiments with specimens having a surface area of 112–156 cm^2. These specimens were reared at 15°C in 200-l aquaria containing natural seawater with a salinity of 37‰. The medium was aerated by bubbling, and it was replaced twice a week. The collected sponges attached to the aquarium bottom in about 10 days.

The sandy materials used in testing sponge selectivity were white polycrystalline quartz with a particle size of 0.25–0.5 mm (BDH laboratory sand); red calcareous sand of the same particle size obtained from the organ-pipe coral *Tubipora musica;* and fragments of a coralline alga, *Lithothamnium* sp., 3–5 mm in size.

To test the differences in behavior between the upper and lower surfaces of the sponge ectosome, a thin layer of a mixture (1:1) of the BDH siliceous and *Tubipora* calcareous sands was laid down on the upper ectosome of five specimens that had attached to the bottom of an aquarium covered by the same mixture. Two experiments were carried out with these specimens. First, cores through the sponge, from upper to lower surface, were inverted and then transplanted, upside-down, inside the same specimen (five replicas). Second, 40 half-cores (20 mm in diameter) were taken from the upper and lower ectosome and reared for 3 months (see Fig. 1).

Observations made with SEM allowed us to distinguish differences in the organization of the two ectosomal surfaces. The samples used for these observations were collected by scuba divers and fixed *in situ* in 10% formalin.

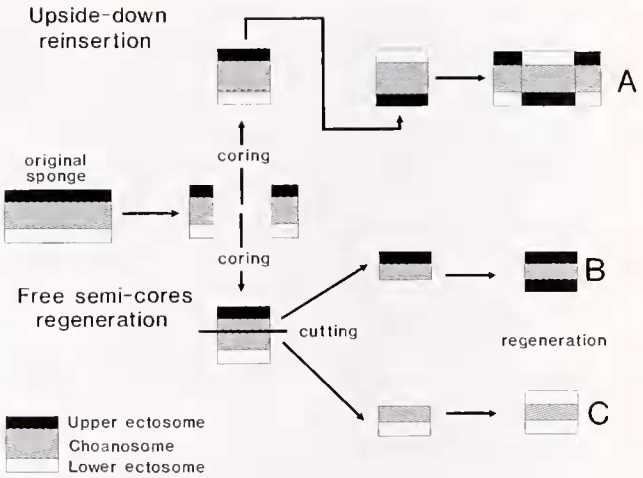

Figure 1. Scheme of coring experiments in *Chondrosia reniformis.* (A) Showing that differences in the mineral selectivity of the upper and lower sides of the ectosome are related to their histology. Cores (about 5–6 cm^2) were cut from the upper to the lower surface and reinserted upside-down in the hole. The central portion of the resulting reconstituted sponges thus had an upper-lower orientation that was reversed. (B, C) Determining whether isolated upper and lower ectosomes can reconstitute an entire animal. Cores were produced and cut transversely to make half-cores. After 2 weeks' regeneration, the upper and lower ectosomes proliferated, enveloping the half-cores.

Results

When a mixture of siliceous and calcareous sand was allowed to settle on the upper ectosome of specimens that had attached to the bottom of the aquarium, only the quartz fragments (white) were incorporated. The calcareous grains (red) were never engulfed; rather they were quickly removed from the sponge surface (Fig. 2a). In contrast, the lower ectosome showed a remarkable preference for incorporating the calcareous grains of the mixture lying on the bottom (Fig. 2b).

The upper ectosome of newly collected specimens that had not yet attached to the aquarium bottom actively incorporated both kinds of minerals (Fig. 2c). Ten days later, after attachment, the upper surface of all the tested specimens began to select siliceous material exclusively.

To verify the difference in mineral selectivity between the two sides of the ectosome, cores (about 5–6 cm^2) extending from the upper to the lower surface were cut from five large specimens and reinserted upside-down, to produce sponges with a portion of their lower surface having an upward orientation (Figs. 1, 2e). A week later, when the explants were perfectly fused in their anomalous positions and the sponges were attached to the aquarium floor, 5 g of *Lithothamnium* calcareous sand was laid on their surface (Fig. 2f). The subsequent behavior of the two types of surface was very different. The upper ectosome (brown) behaved normally, quickly removing calcareous particles (Fig. 2g–h); but the inverted, originally lower

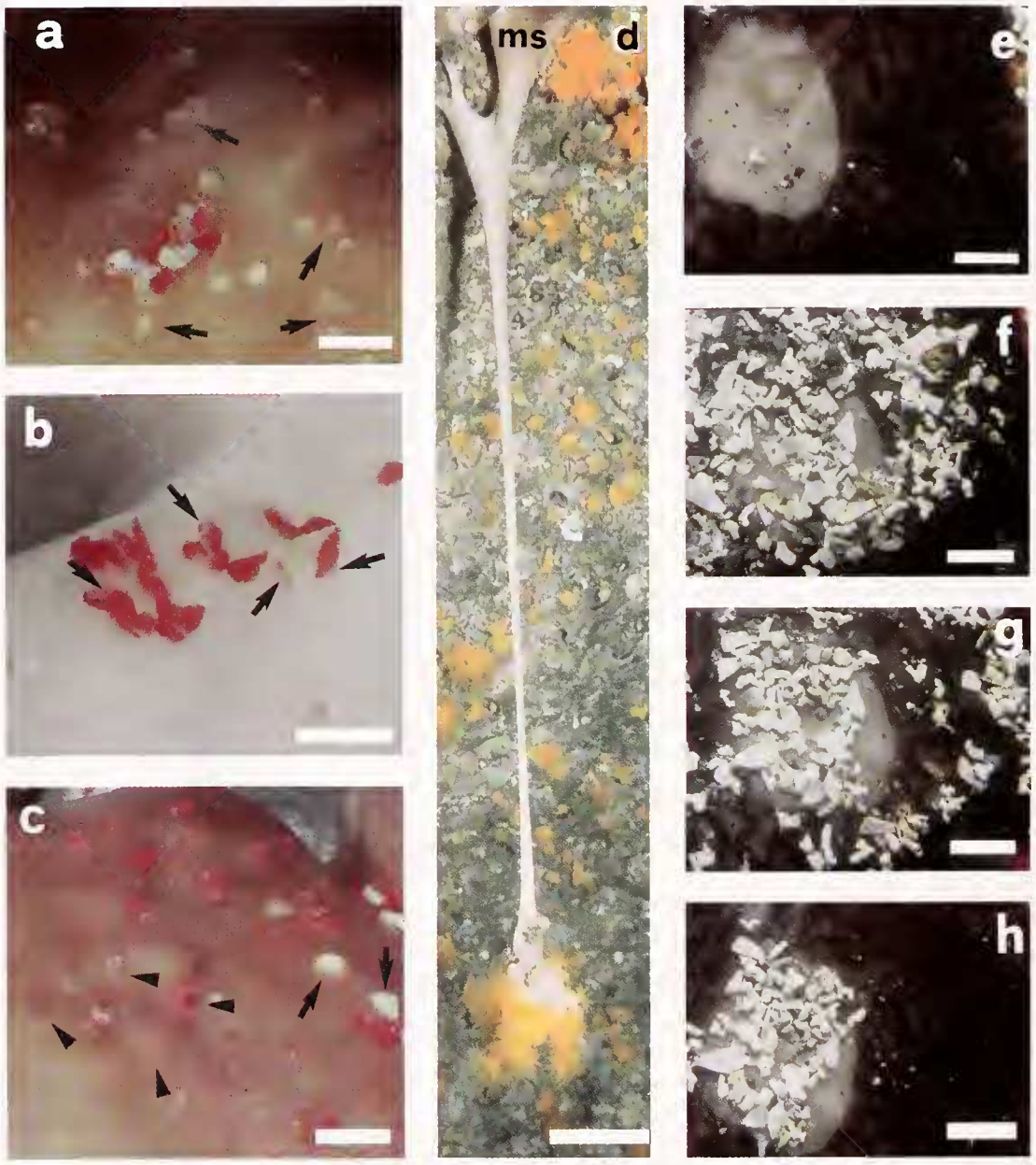

Figure 2. Mineral selectivity experiments. A 1:1 mixture of calcareous (red) and quartz (white) grains were allowed to settle on the upper and lower ectosome of *Chondrosia reniformis* specimens. (a) The upper ectosome (brown) has incorporated several quartz grains (arrows), while almost all the calcareous particles have been eliminated. (b) The opposite happens in the lower ectosome (white), which incorporates the calcareous fraction. Arrows indicate the thin collagenous sheet growing on the calcareous fragments. (c) The upper ectosome of a free, unattached specimen incorporates both quartz (arrows) and carbonatic particles (arrow heads). (d) Asexual reproduction in *C. reniformis*. The underwater photograph shows a long (1 m), stretched filamentous outgrowth hanging from a mother sponge (ms) that lives attached to the vault of a cave. A new sponge has formed at the free tip of the filament. (e) A core (about 6 cm^2) cut and newly inserted upside-down in a large specimen of *C. reniformis*. When the explant was perfectly fused in its anomalous position, and the sponge had attached to the aquarium floor, calcareous grains were laid on its surface (f). Afterwards, the behavior of the two portions was very different. The upper surface (brown) behaves normally, quickly removing calcareous particles; shown at 24 h (g) and 48 h (h). The section that was originally the lower surface (whitish) did not move the particles. Scale bars: a–c = 1 mm; d = 10 cm; e–h = 1 cm.

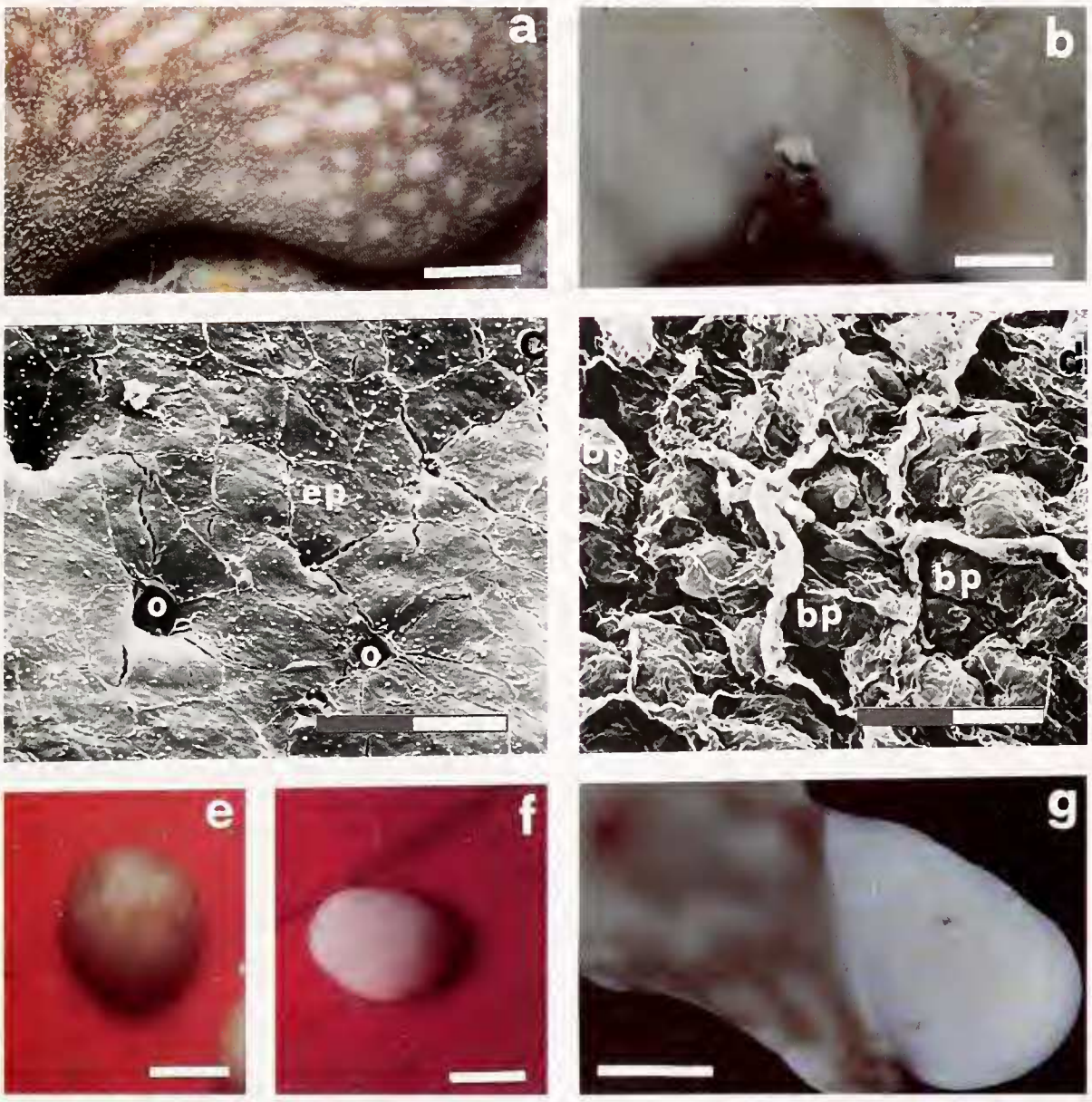

Figure 3. (a) The upper ectosome of *Chondrosia reniformis*, brown in color due to the presence of numerous melanocytes, and (b) the lower whitish one. SEM observations reveal differences between the two zones. (c) The upper surface is covered by polygonal flattened exopinacocytes (ep) and pierced by the incurrent openings, or ostia (o). (d) The lower surface shows no pores, and the basopinacocytes (bp) are covered by a collagenous sheet. Twelve weeks after preparation, the half-cores contain only an upper (e) or lower (f) ectosome. Two half-cores (g) derived from the upper (left) and lower (right) ectosomes, brought together and fused maintaining the characteristics of their original position. Scale bars: a–b, e–g = 1 cm; c–d = 50 μm.

surfaces (whitish) did not move the particles, and 3 months later, they were all incorporated.

The gross morphological differences between the upper (Fig. 3a) and lower surfaces of the sponge (Fig. 3b) were supported by SEM observations: the upper ectosome is entirely covered by polygonal flattened pinacocytes and perforated by the incurrent openings (*ostia*) (Fig. 3c), whereas the pinacoderm of the lower surface is covered by a collagenous sheet that lacks ostia (Fig. 3d).

To verify that the polarization between the upper and lower ectosome arises very early in development, experiments were performed with half-cores; these are fractions of sponge tissue that contain a portion of choanosome covered on one side by either upper or lower ectosome

(see Fig. 1). In the first 2 weeks of culture, the ectosome of both kinds of free half-cores actively proliferated, and the pieces assumed a spherical shape (Fig. 3e–f). The half-cores covered with lower ectosome (Fig. 3f) settled on the smooth bottom of the aquarium in 3–4 weeks, whereas those with the upper ectosome (Fig. 3e) settled after about 10–12 weeks. Furthermore, 3 months after the beginning of the experiment, the half-cores with lower ectosome were covered by a collagenous, non-cellularized layer that remained white and formed no osculum; the half-cores with the upper ectosome produced a new oscule in 12–15 weeks and showed a normal pinacoderm perforated by pores. Sometimes, when half-cores deriving from the upper and lower ectosome came in contact, they fused together; but a normal sponge was never reconstituted, although the two kinds of ectosome remained distinct on the opposite sides of the sponge (Fig. 3g).

Discussion

The structural and functional differences between the two sides of the ectosome of the sponge *Chondrosia reniformis* suggest a strong polarization—upper pole *versus* lower pole—along the axis of the sponge. The activity of the upper ectosome is likely due to the pinacocyte-mineral interaction. More problematic is the basis for the preferences of the lower ectosome for calcareous substrata. In other sponges, a nonspecific attachment to the substratum is probably due to the secretion by the basopinacocytes of a complex basal lamella (Pavans de Ceccatty, 1981) that anchors the sponge but prevents any contact between the cells and the substratum.

The ability of the upper ectosome to discriminate between silica and carbonates is present only in attached specimens and vanishes in free, nonattached ones, which incorporate both materials indiscriminately. This distinction is interpretable if we consider the asexual reproductive strategy of the species: Sponges living on overhanging ledges or on the vaults of submarine grottos give rise to long, thin pendant filaments (Fig. 2d). Cell reorganization within the apical region of these filaments produces a new, functional, but suspended animal. When the filament breaks, the bud is separated from the maternal sponge (Gaino *et al.*, 1995); it falls and must attach quickly irrespective of the side of the ectosome that comes in contact with the substratum. This behavior indicates, not only that mineral receptors are distributed evenly on the sponge surface, but also that these receptors may be activated or deactivated under particular conditions by an environmental switch. We suppose that the mineral discrimination of the upper ectosome is switched on by the adhesion of the sponge to the bottom.

Our studies indicate two modalities of mineral incorporation that are associated with the two sides of the ectosome. The upper side collects quartz and silicates, which strengthens the collagenous structure; this is a dynamic process comprising incorporation of the particles and resizing of the quartz grains, with their elimination *via* the aquiferous system (Bavestrello *et al.*, 1995). The lower side specifically engulfs the calcareous substrata, thus fixing the sponge to the bottom.

There is a rich literature about the very wide potential for cytodifferentiation in sponges (see Simpson, 1984). Our data indicate that, at least in *C. reniformis*, the morphological differences between the upper and lower regions of the ectosome are sharp, probably arising in a very early stage of sponge ontogeny; that is, a functionally complete specimen cannot be reconstituted from a portion of only upper or lower ectosome with its adjacent choanosome. Connes (1966, 1968) has demonstrated that the ectosome and choanosome of *Tethya aurantium* have different potentials for reconstructing an entire sponge, but our data provide the first indication of differences in this process associated with distinct zones of the same ectosome.

In higher metazoans, the spatiotemporal development of morphological structures is regulated by homeobox genes (Lawrence, 1992). These genes have also been observed in lower metazoans such as sponges (Kruse *et al.*, 1994; Coutinho *et al.*, 1994; Degnan *et al.*, 1995; Seimiya *et al.*, 1997), but their meaning has been obscure until now. We hypothesize that the acquisition of an axial polarity in the sponge may be controlled by these genetic structures.

Acknowledgements

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